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Tubeless microfluidic angiogenesis assay with three-dimensional endothelial-lined microvessels

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ABSTRACT

The study of angiogenesis is important to understanding a variety of human pathologies including cancer, cardiovascular and inflammatory diseases. In vivo angiogenesis assays can be costly and time-consuming, limiting their application in high-throughput studies. While traditional in vitro assays may overcome these limitations, they lack the ability to accurately recapitulate the main elements of the tissue microenvironment found in vivo, thereby limiting our ability to draw physiologically relevant biological conclusions. To bridge the gap between in vivo and in vitro angiogenesis assays, several microfluidic methods have been developed to generate in vitro assays that incorporate blood vessel models with physiologically relevant three-dimensional (3D) lumen structures. However, these models have not seen widespread adoption, which can be partially attributed to the difficulty in fabricating these structures. Here, we present a simple, accessible method that takes advantage of basic fluidic principles to create 3D lumens with circular cross-sectional geometries through ECM hydrogels that are lined with endothelial monolayers to mimic the structure of blood vessels in vitro. This technique can be used to pattern endothelial cell-lined lumens in different microchannel geometries, enabling increased flexibility for a variety of studies. We demonstrate the implementation and application of this technique to the study of angiogenesis in a physiologically relevant in vitro setting.

1. Introduction

Angiogenesis, the neovascularization of blood vessels from preexisting vasculature, is an important biological process involved in normal growth and development, as well as in various human pathologies including cancer, cardiovascular diseases, and inflammatory disorders. In cancer specifically, angiogenesis is necessary for tumors to grow beyond a critical size of a few millimeters. Without new vessel formation and proper blood supply, tumor cells too distant (>~200 μm) from existing vessels would lack the supply of oxygen and nutrients essential for cell survival and proliferation [1]. Because of the importance of angiogenesis in tumor growth, metastasis, and overall cancer progression, therapeutic strategies have been developed around the concept of inhibiting angiogenesis with drugs and other angiostatic agents to restrict blood supply to the tumor. This is an area in drug discovery that continues to undergo intense study [2,3]. The ability to study angiogenesis and investigate the effects of various factors on angiogenic responses is thus critical for furthering our understanding of the mechanisms of cancer development, as well as for the development of new and effective therapies.

Current angiogenesis assays span a wide range of methods that include in vivo preparations, organ cultures, and in vitro assays [4]. While in vivo methods such as the popular cranial window and dorsal skin chamber preparations have been instrumental in providing deep insights into the angiogenic process, these preparations are time-consuming, labor-intensive, expensive, and require significant skill in surgery, and thus are unsuitable as routine assays for widespread adoption or for high-throughput testing. Organ cultures such as the aortic ring and chick aortic arch assays are simpler preparations than in vivo methods, and maintain important elements of the complex tissue microenvironment, but tissue isolation, culture, and explant outgrowth of aortas can be time-consuming, challenging to do repeatedly and consistently, and difficult to scale up [5]. Thus, for high-throughput applications such as screening of large drug compound libraries and combinatorial testing of cellular and extracellular factors, a more suitable approach is to employ in vitro assays that rely on

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simple, accessible cell cultures and readily available substrates, and circumvent laborious lab procedures that involve the handling of animals and tissue explants. However, an often-cited major shortcoming of current in vitro assays is their inability to accurately recapitulate the main elements of the tissue microenvironment found in vivo, and this issue limits our ability to draw accurate biological conclusions. Therefore, an urgent need exists for the development of improved in vitro angiogenesis assays that can continue to offer high-throughput capacity and simple convenient operation while significantly enhancing the physiological relevance of the in vitro tissue microenvironment.

Recently, microfluidics technology has been applied to improve the spatiotemporal control of the cell or tissue microenvironment [6], thereby enabling the development of new and useful cell-based assays for biological research [7–10]. In particular, microfluidics has provided significant advances in new models of vascular function [11–15], with several notable designs specifically tailored to study the processes associated with angiogenesis [16,17]. While these methods have demonstrated the potential benefits of working at the microscale, and clearly offer more sophistication for studying angiogenesis, there remain significant challenges related to the fabrication and maintenance of blood vessel mimics that closely resemble three-dimensional vessel lumens in vivo. Furthermore, it is unclear whether these systems can be easily scalable for applications requiring increased throughput.

Here, we present a simple, accessible method that takes advantage of basic fluidic principles to create three-dimensional (3D) lumens with circular cross-sectional geometries lined with patent 3D endothelial monolayers applied to study angiogenesis in physiologically relevant in vitro microenvironments. The method requires only the use of a micropipette, and has the potential to be scaled into large arrays interfaced with automated liquid handlers as previously shown [18–20]. The method also allows incorporation of other cell types for organ-like cocultures that mimic the structure and organization of blood vessel lumens in vivo. We describe the development and application of 3D endothelial-lined lumens (ELL) with proper barrier function, biological response to VEGF gradients in the form of angiogenic sprouting, and further demonstrate the flexibility of the method for generating vessel networks on demand, without the need for re-designing molds from scratch.

2. Materials and methods

2.1. Device fabrication

Polydimethylsiloxane (PDMS, Sylgard 184 Silicone Elastomer Kit, Dow Corning, Midland, MI) elastomer base and curing agent were mixed at a 10:1 ratio and degassed for 45 min under vacuum at room temperature. The degassed PDMS was then poured over SU-8 master molds that were generated using standard soft lithography methods [21]. PDMS was cured at 80 °C for 4 h. Microchannel geometries used in these experiments varied depending on the application (Fig. 1).

2.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza (Walkersville, MD, USA) and maintained with endothelial growth medium (EGM-2) with Bullet Kit (EGM-2MV; Lonza, Walkersville, MD, USA) on regular tissue culture flasks pre-coated with 2 μg/cm^2 fibronectin (FN) (Sigma–Aldrich, St. Louis, MO,

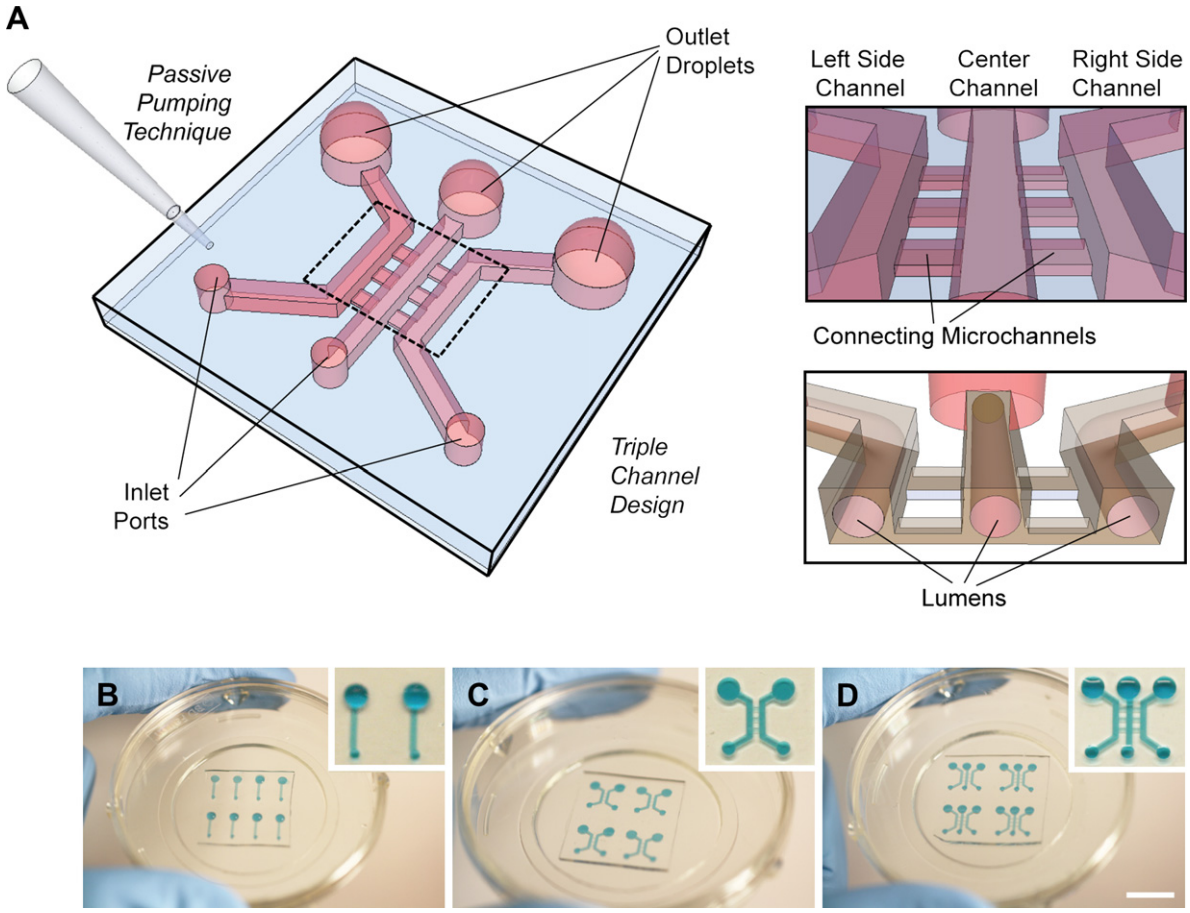

Fig. 1. Passive pumping-based microfluidic angiogenesis assay with 3D cylindrical lumens. (A) Illustration of a triple channel design with connecting microchannels. (B–D) Microchannel systems can be (B) single, (C) double, or (D) triple channel designs, and are arrayable. Devices are plasma-treated and bonded to glass-bottom Petri dishes. Scale bar ~10 mm.